Figure 1

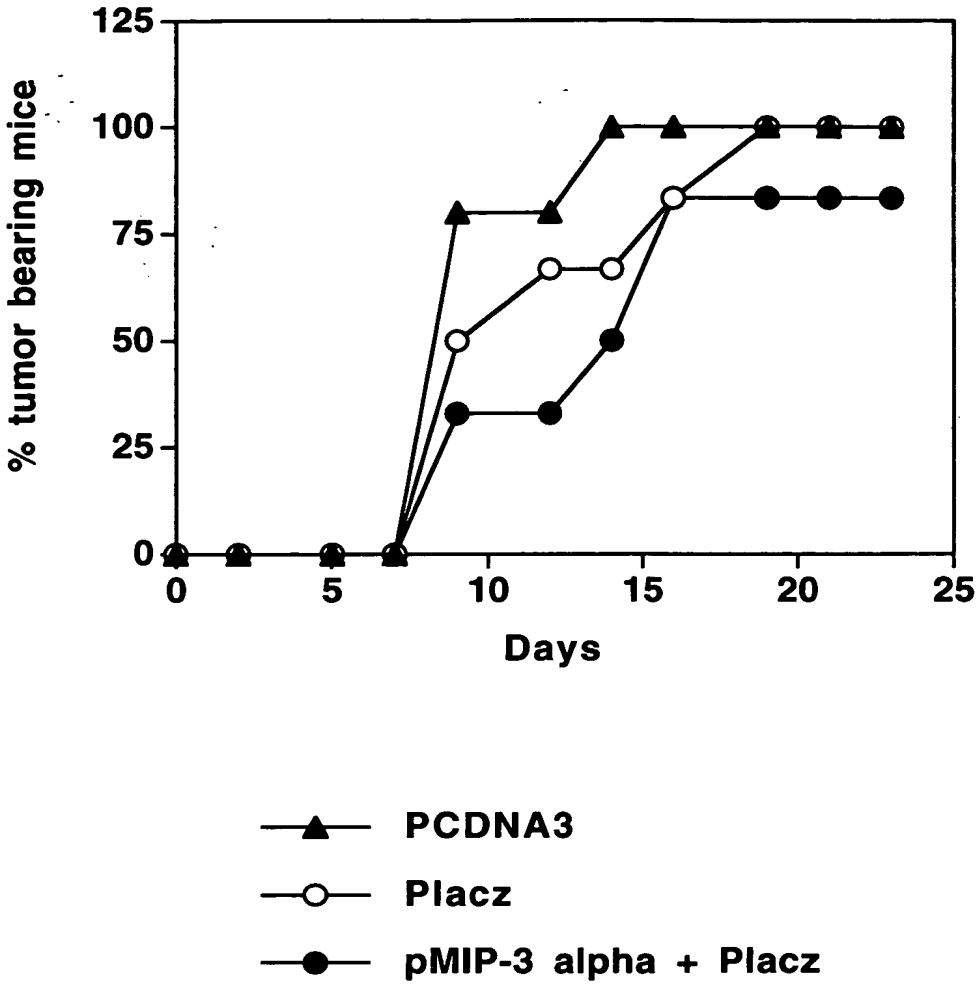
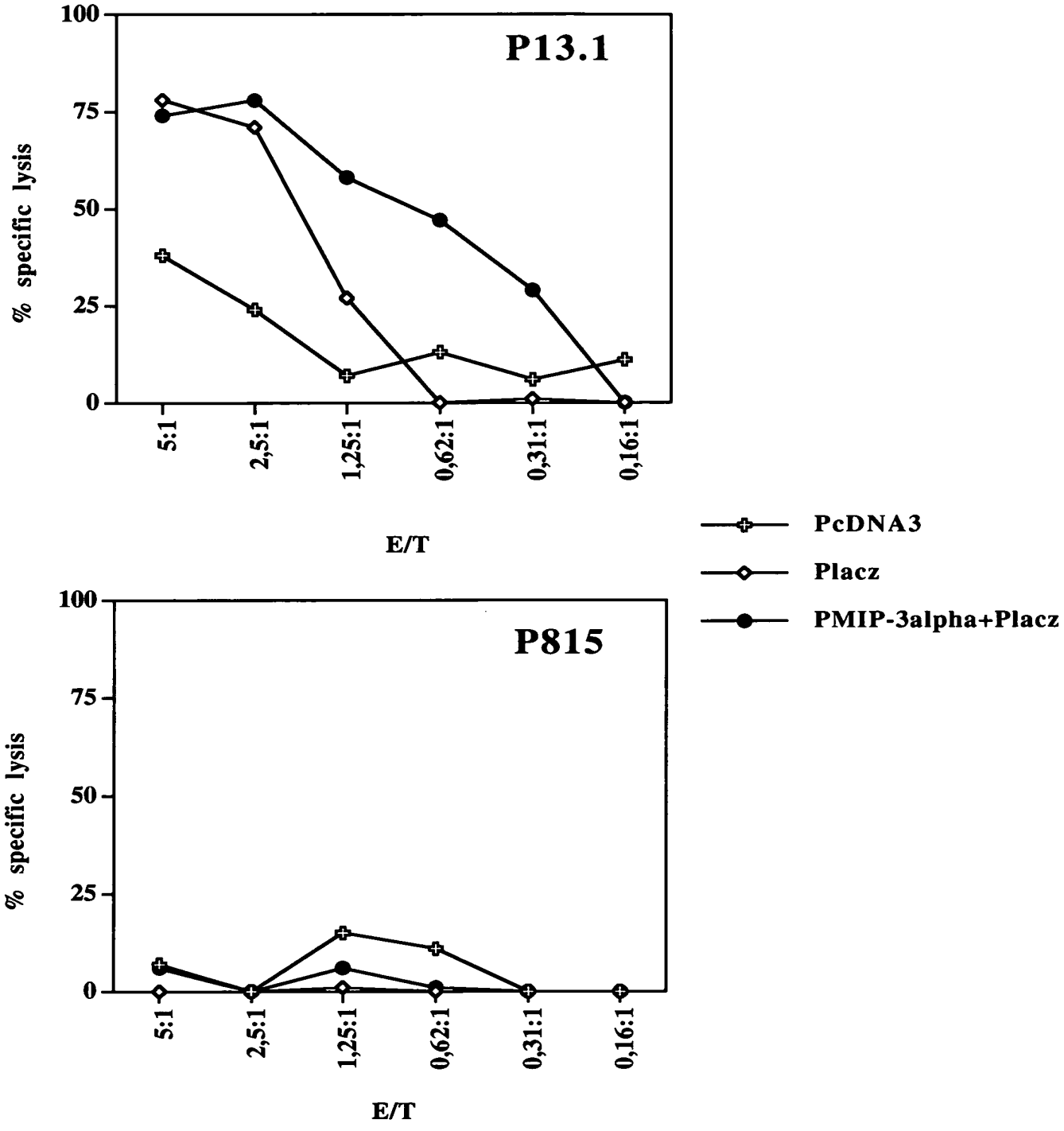

125
100
75
50
25
0
% tumor bearing mice
0
5
10
15
20
25
Days
PCDNA3
Placz
pMIP-3 alpha + Placz

Figure 2


P13.1
100
75
50
25
0
% specific lysis
5:1
2,5:1
1,25:1
0,62:1
0,31:1
0,16:1
E/T
P815
100
75
50
25
0
% specific lysis
5:1
2,5:1
1,25:1
0,62:1
0,31:1
0,16:1
E/T
PcDNA3
Placz
PMIP-3alpha+Placz

hMCP-4 chemokine

- Nucleotide sequence (coding only)

ATGAAAGTCTCTGCAGTGCTTCTGTGCCT
GCTGCTCATGACAGCAGCTTTCAACCCCC
AGGGACTTGCTCAGCCAGATGCACTCAA
CGTCCCATCTACTTGCTGCTTCACATTTA
GCAGTAAGAAGATCTCCTTGCAGAGGCT
GAAGAGCTATGTGATCACCACCAGCAGG
TGTCCCCAGAAGGCTGTCATCTTCAGAAC
CAAACTGGGCAAGGAGATCTGTGCTGAC
CCAAAGGAGAAGTGGGTCCAGAATTATA
TGAAACACCTGGGCCGGAAAGCTCACAC
CCTGAAGACTTGA

- Amino acid sequence (leader sequence not present in recombinant protein in italics)

*MKVSAVLLCLLLMTAAFNPQGLA*QPDALNV
PSTCCFTFSSKKISLQRLKSYVITTSRCPQK
AVIFRTKLGKEICADPKEKWVQNYMKHL
GRKAHTLKT

1 µg 2.5 µg 4 µg

29 kDa →
19.2 kDa →
7.5 kDa →

← h MCP-4 (8.6 kDa)

SDS-PAGE (18%) and silver staining of human recombinant MCP-4

Figure 3

(A) Local recruitment of CD11b+ cells 2 h following hMCP-4 injection
(B) Increase of dendritic cells in the draining lymph node 20 hours after hMCP-4 s.c. injection: absolute numbers. Right panel statistical difference between hMCP-4 and controls p< 0.01 (Student's t test)

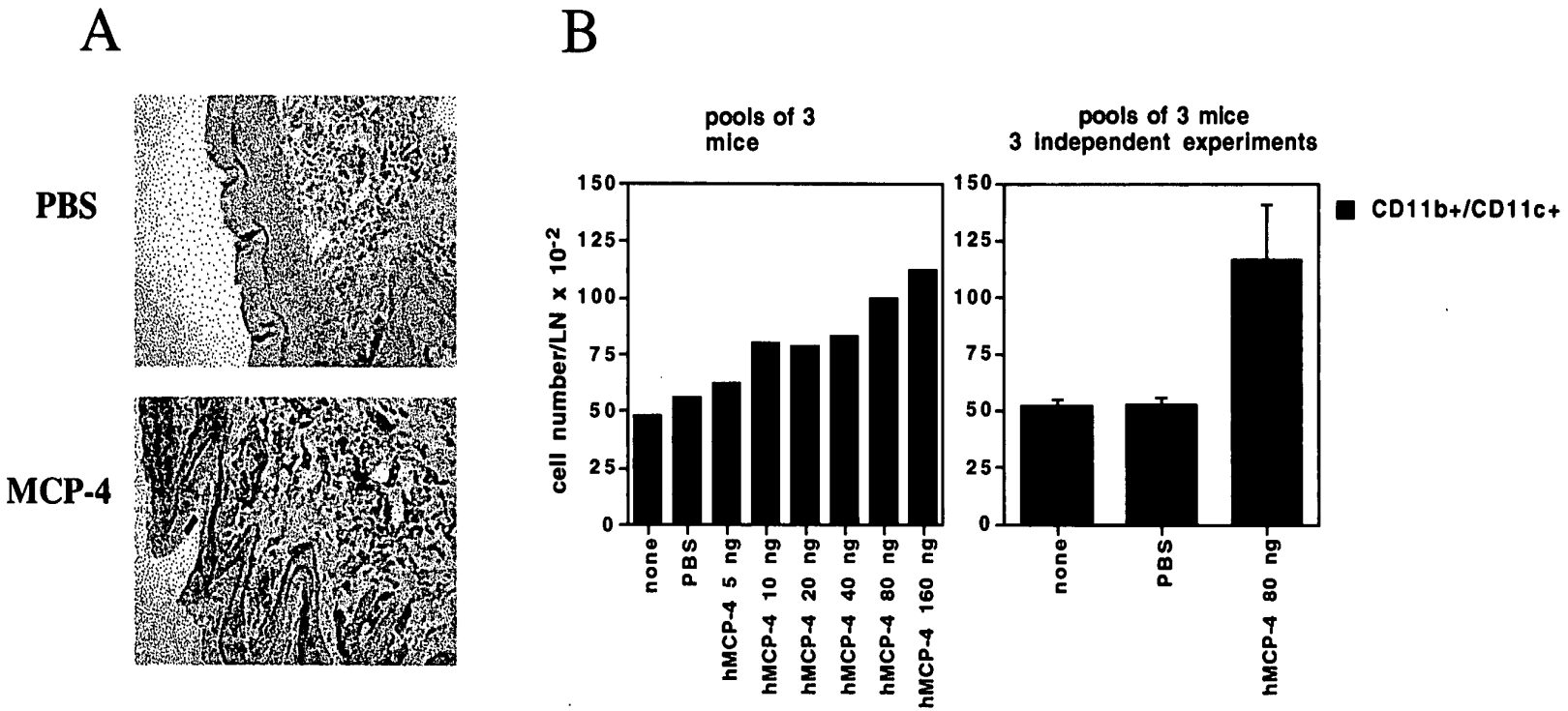
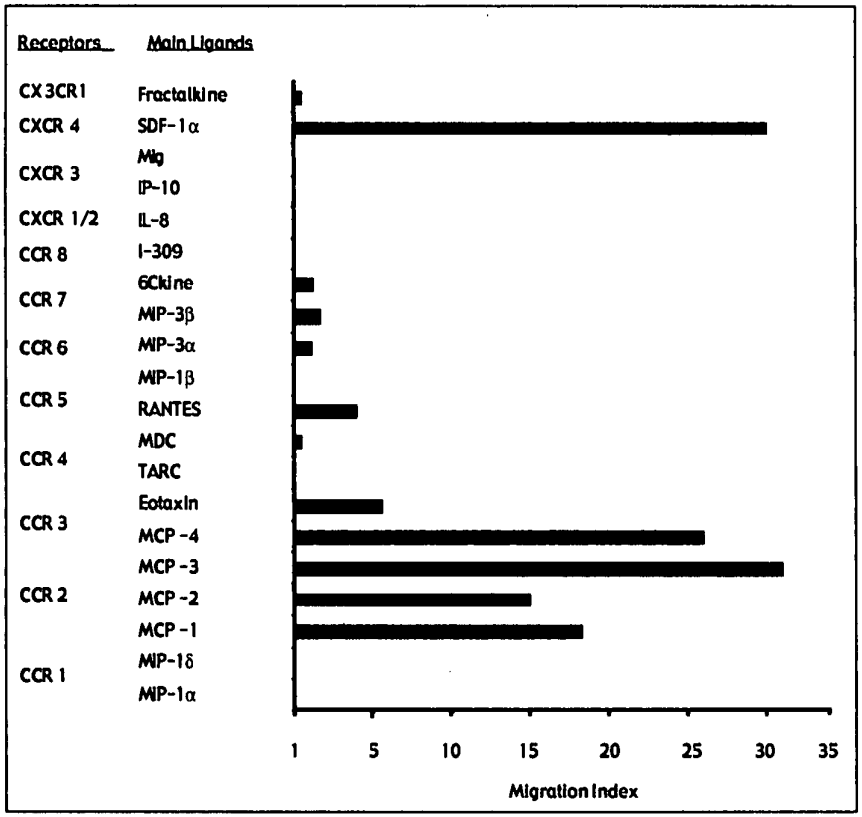
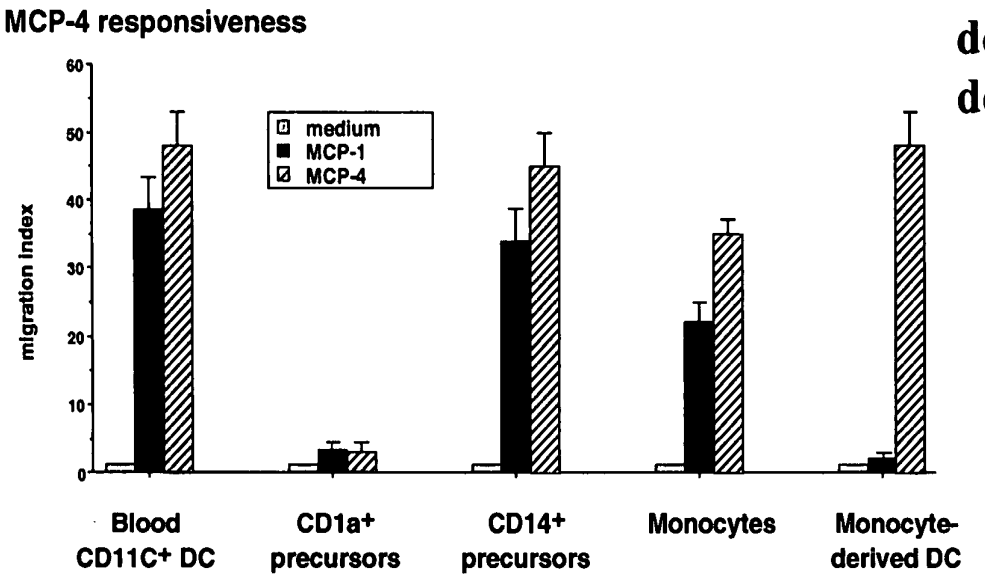


Figure 4



Human MCP-4 is one of the most potent chemokine active on human dendritic cells isolated from blood



Human MCP-4 is active on blood dendritic cells and monocyte-derived dendritic cells, unlike hMCP-1

Figure 5

MCP-4 injection increases the antigen-specific humoral response following beta-galactosidase DNA immunization (50 micrograms DNA injection 3 hours after 100 ng hMCP-4 injection in rear right footpad)
Figure shows anti-betagalactosidase antibodies measured after 4 immunizations significance hMCP-4 + pLacz compared with PBS + pLacz : Student's t test

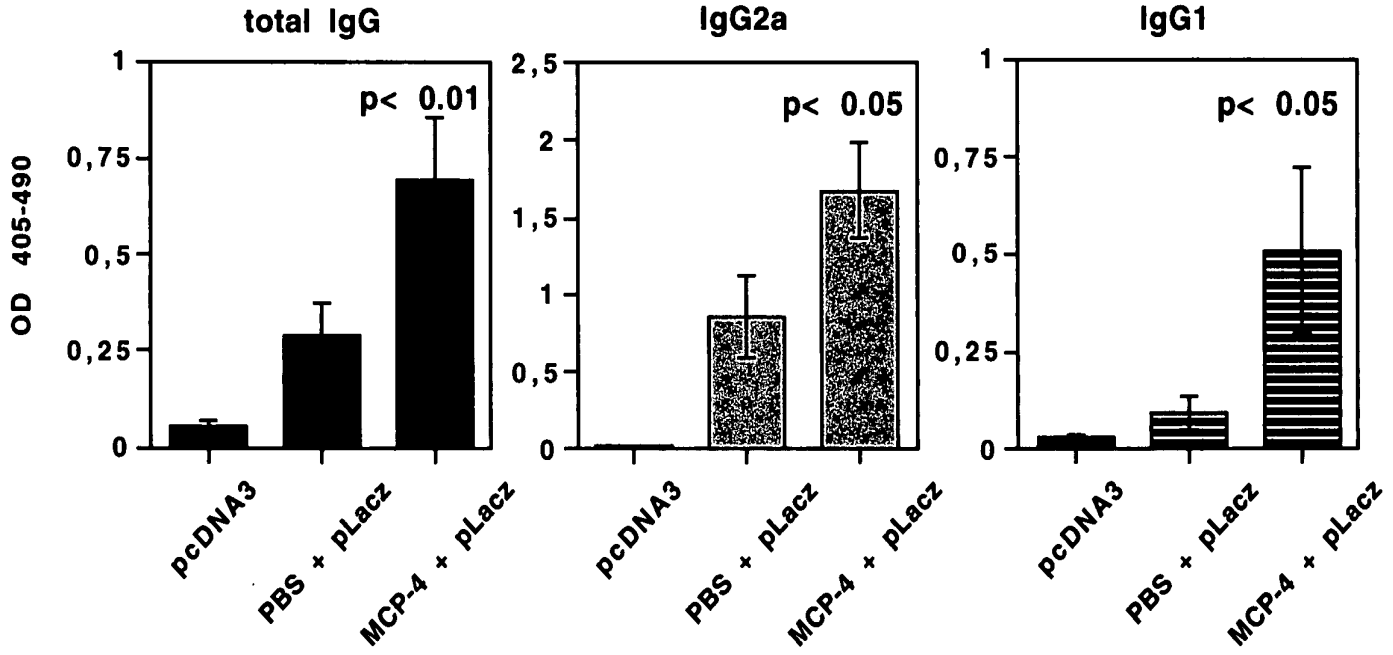


Figure 6

MCP-4 injection increases the anti-tumor effect induced by beta-galactosidase DNA immunization (50 micrograms DNA injection 3 hours after 100 ng hMCP-4 injection in rear right footpad, four immunizations prior to tumor challenge) when mice are challenged with a C26 colon carcinoma cell line that expresses beta-galactosidase significance hMCP-4 + pLacz compared with PBS + pLacz : $p<0.05$ logrank MCP-4 opp: hMCP-4 injected at distant site

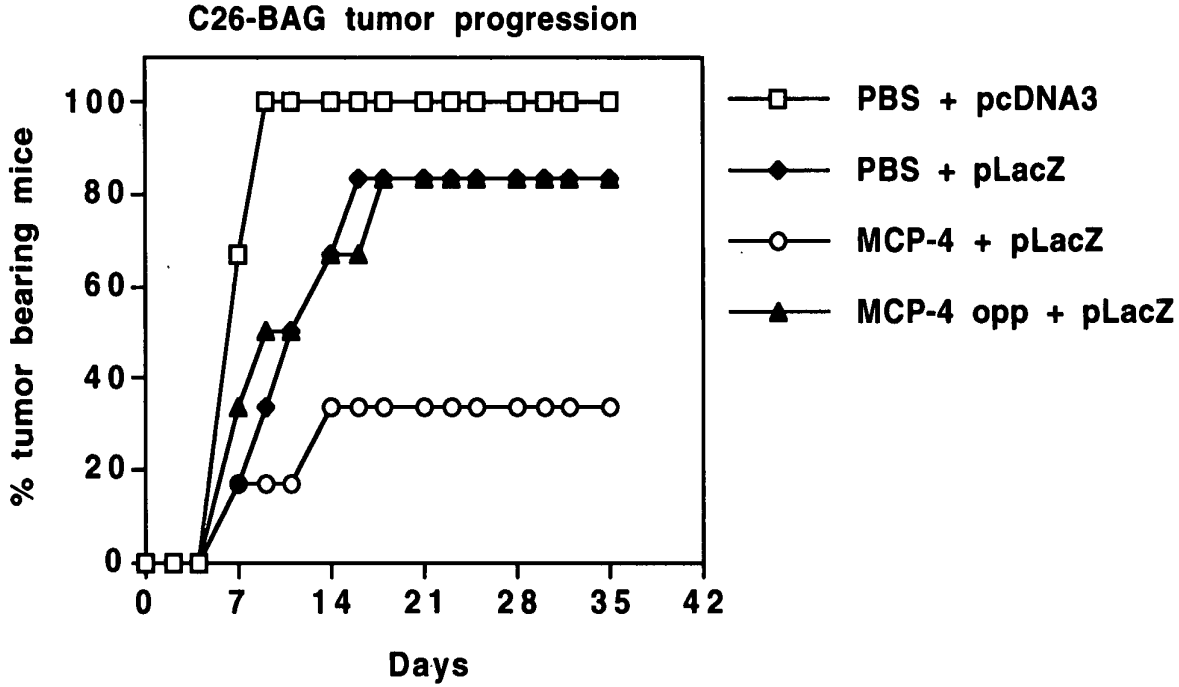
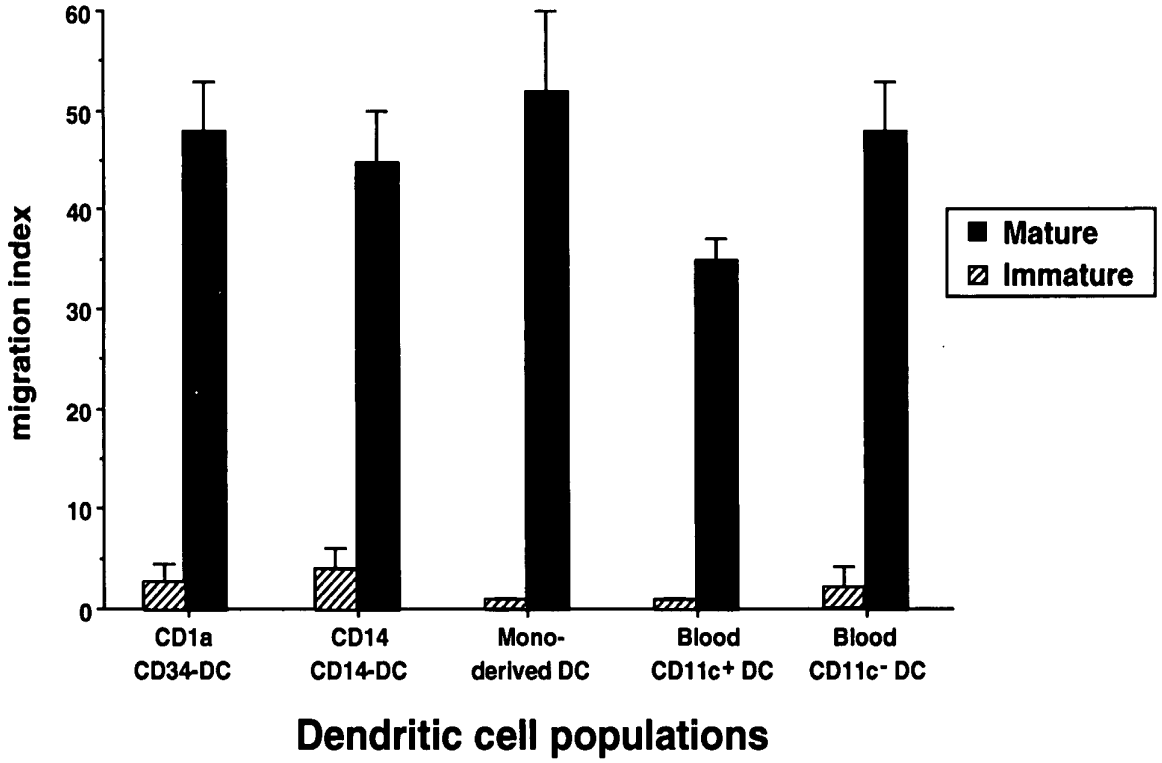


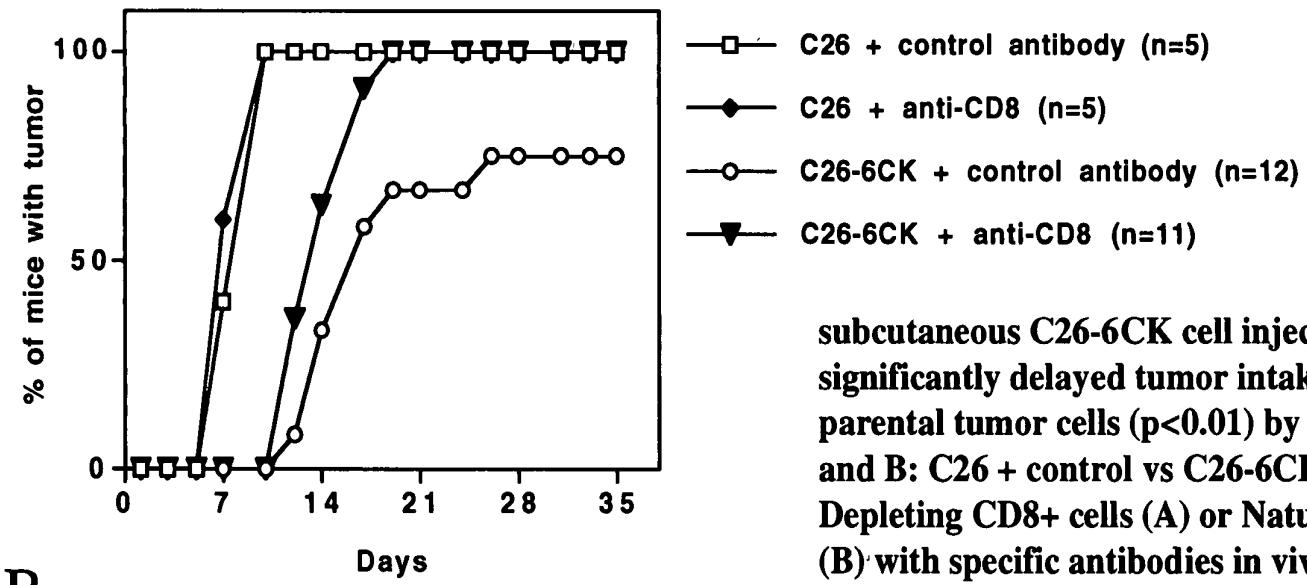
Figure 7



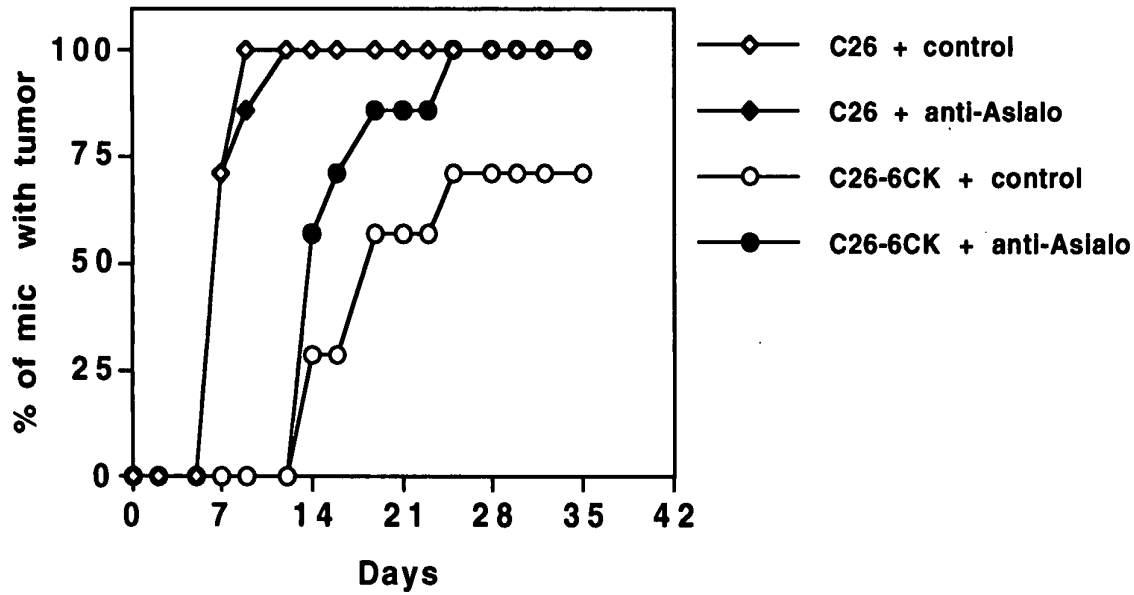
Human 6Ckine is a chemotactic factor for all subsets of human dendritic cells, derived in vitro or isolated ex vivo.

Figure 8

A

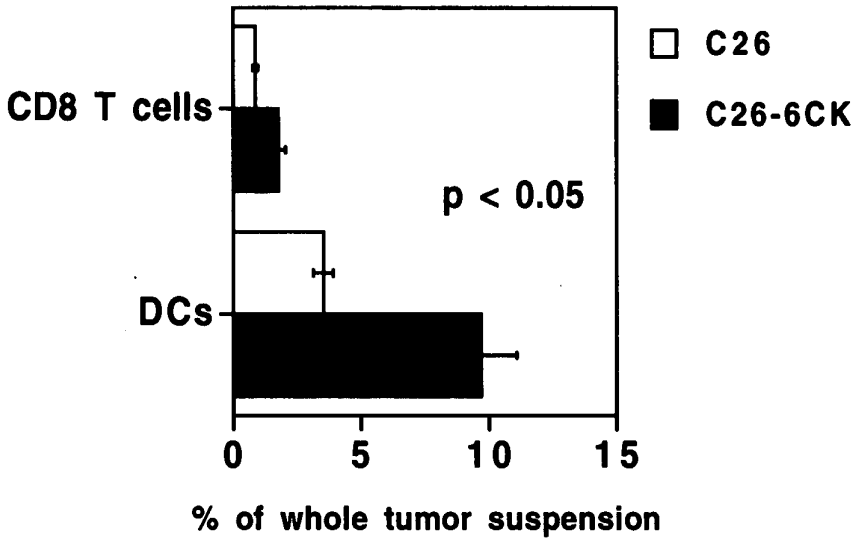


B



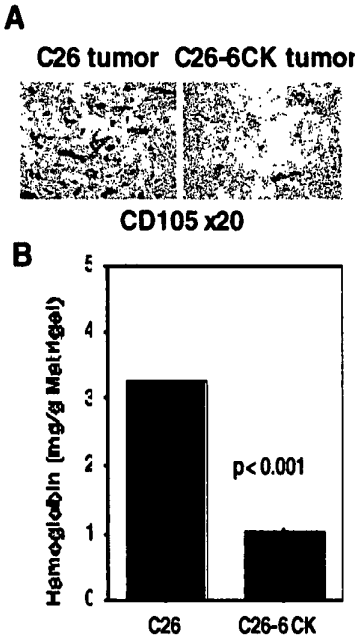
subcutaneous C26-6CK cell injection results in significantly delayed tumor intake compared to parental tumor cells ($p<0.01$) by logrank analysis (A and B: C26 + control vs C26-6CK + control). Depleting CD8+ cells (A) or Natural Killer cell activity (B) with specific antibodies in vivo partially reverts the delayed tumorigenicity of the C26-6CK tumor cells, indicating that CD8+ cells and NK cells play a role in delaying tumor growth.

Figure 9



C26 wild-type tumors or C26-6CK tumors expressing m6Ckine have been analyzed for CD8 T cells and CD11c+MHC classII+ dendritic cell (DC) infiltration by flow cytometry analysis of whole tumor suspension (n=7).
Data show a significant recruitment of both leukocyte subsets in C26-6CK tumors compared to C26 tumors (Student's t test).

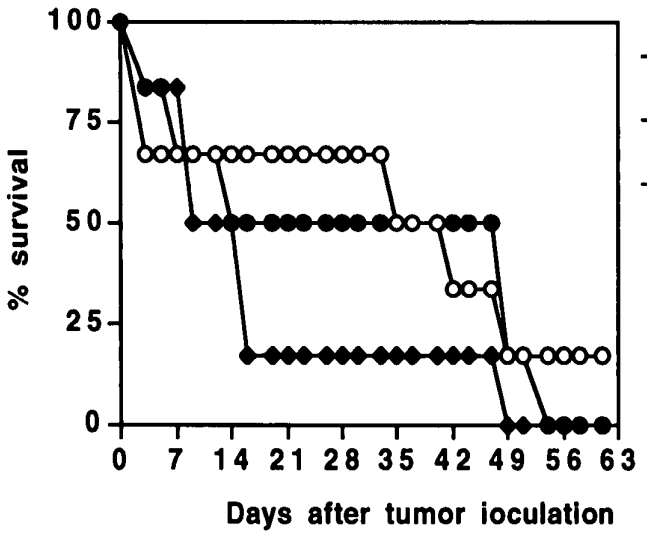
Figure 10



C26 wild-type tumors or C26-6CK tumors expressing m6Ckine have been analyzed for the development of blood vasculature (CD105 staining, A) or angiogenic potential in a Matrigel assay (B).
Data show a significant decrease of angiogenesis induced by m6Ckine gene transfer into the C26 tumor.

Figure 11

A



treatment: D12-D37

Mice (n=6 / group) were inoculated s.c. with C26 tumor cells at Day 0, randomized and treated with 10 ng recombinant m6Ckine or h6Ckine or PBS intra-tumor at Days 12, 13, 14, 15, 18, 21, 23, 25, 28, 30, 32, 35, 37.
Mice injected with h6Ckine and m6Ckine show improvement in survival compared with PBS vehicle alone (A).
Injection of h6Ckine also decreased the growth of tumors (B).
Note: data on tumor surface obtained from surviving mice.

B

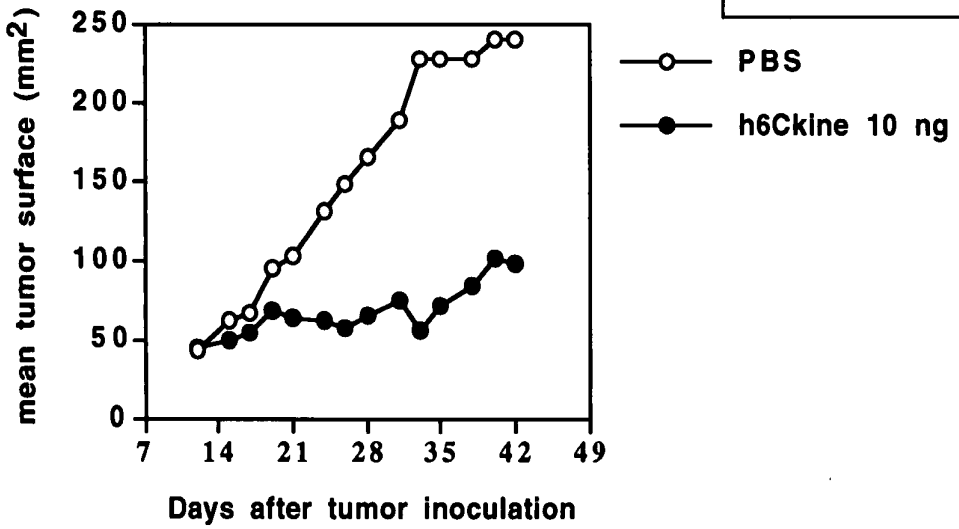
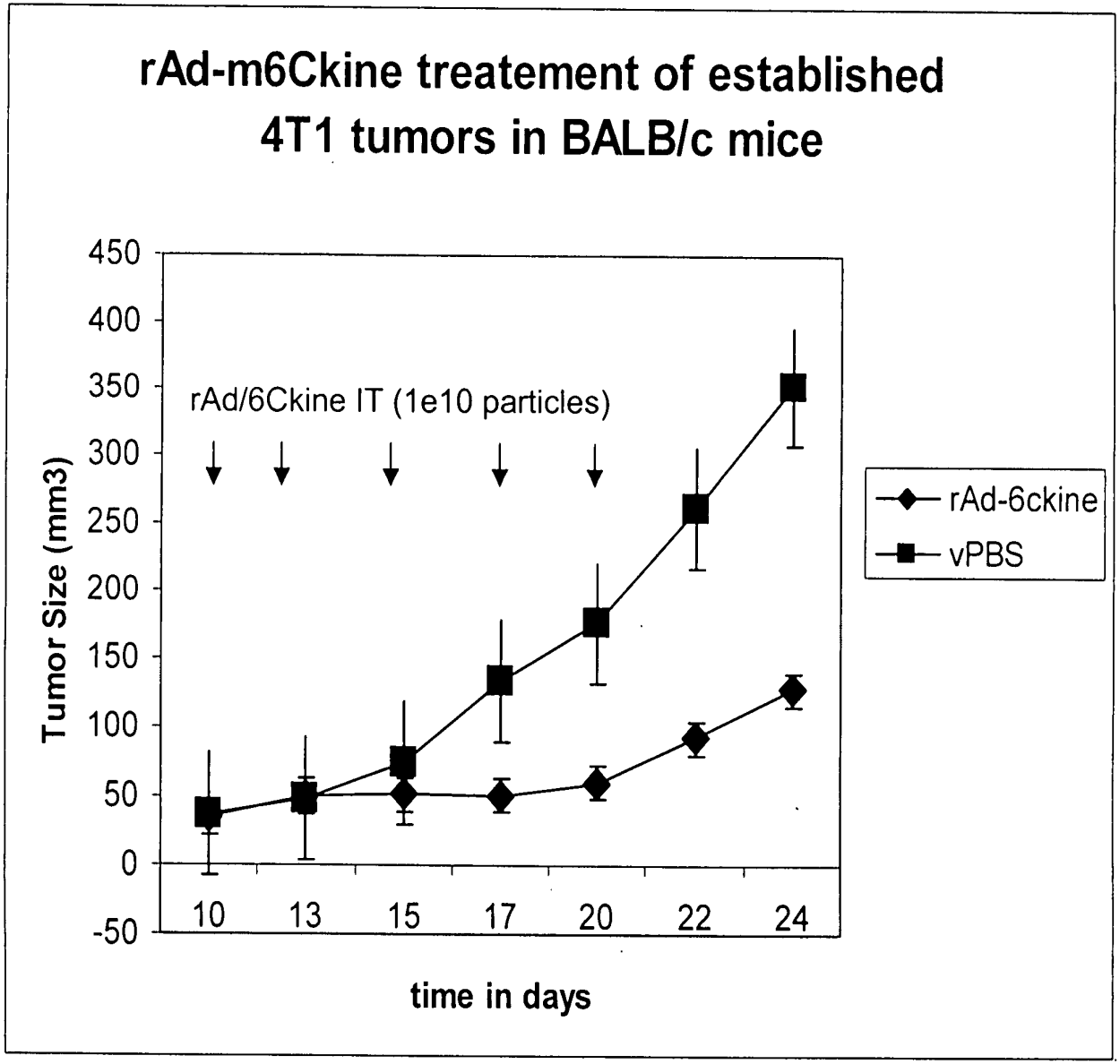


Figure 12


rAd-m6Ckine treatement of established
4T1 tumors in BALB/c mice
rAd/6Ckine IT (1e10 particles)
Tumor Size (mm3)
450
400
350
300
250
200
150
100
50
0
-50
10
13
15
17
20
22
24
time in days
rAd-6ckine
vPBS